

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Levitsky et al.

Application No. Unassigned

Filed: November 16, 2001

Art Unit: Unassigned

Examiner: Unassigned

For: A UNIVERSAL  
IMMUNOMODULATORY CYTOKINE-  
EXPRESSING BYSTANDER CELL  
LINE AND RELATED COMPOSITIONS  
AND METHODS OF MANUFACTURE  
AND USE

PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

1. A universal bystander cell line, which:
  - (i) is a human cell line,
  - (ii) naturally lacks major histocompatibility class I (MHC-I) antigens and major histocompatibility class II (MHC-II) antigens, and
  - (iii) is modified by introduction of a nucleic acid molecule comprising a nucleic acid sequence encoding granulocyte macrophage-colony stimulating factor (GM-CSF) operably linked to a promoter,  
wherein said universal bystander cell line expresses about 500 ng or greater GM-CSF/ $10^6$  cells/24 hours.
2. The universal bystander cell line of claim 1, wherein said human cell line is characterized by the absence of B-lymphocyte markers of immunoglobulin, an Epstein-Barr virus (EBV) genome and an associated nuclear antigen, and receptors for EBV.
3. The universal bystander cell line of claim 1, wherein said human cell line is derived from a blast crisis of chronic myeloid leukemia.
4. The universal bystander cell line of claim 1, wherein said human cell line is K562.
5. The universal bystander cell line of claim 1, which expresses about 1,000 ng or greater GM-CSF/ $10^6$  cells/24 hours.

6. The universal bystander cell line of claim 1, which grows in defined medium.
7. The universal bystander cell line of claim 1, wherein said promoter is a cytomegalovirus promoter.
8. The universal bystander cell line of claim 4, which expresses about 1,000 ng or greater GM-CSF/10<sup>6</sup> cells/24 hours.
9. The universal bystander cell line of claim 4, which grows in defined medium.
10. The universal bystander cell line of claim 4, wherein said promoter is a cytomegalovirus promoter.
11. The universal bystander cell line of claim 1, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising about 400 µg/ml or greater hygromycin.
12. The universal bystander cell line of claim 11, wherein said universal bystander cell line is selected by growth in a culture medium comprising about 1,000 µg/ml or greater hygromycin.
13. The universal bystander cell line of claim 4, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising about 400 µg/ml or greater hygromycin.
14. The universal bystander cell line of claim 13, wherein said universal bystander cell line is selected by growth in a culture medium comprising about 1,000 µg/ml or greater hygromycin.
17. A composition comprising the universal bystander cell line of claim 1 and a cancer antigen.

18. A composition comprising the universal bystander cell line of claim 2 and a cancer antigen.

19. A composition comprising the universal bystander cell line of claim 4 and a cancer antigen.

20. A composition comprising the universal bystander cell line of claim 5 and a cancer antigen.

21. A composition comprising the universal bystander cell line of claim 8 and a cancer antigen.

22. A method of making a universal GM-CSF-expressing bystander cell line, which method comprises:

(i) obtaining a human cell line that lacks MHC-I antigens and MHC-II antigens;  
(ii) modifying said human cell line by introducing into said human cell line a nucleic acid molecule comprising a nucleic acid sequence encoding GM-CSF operably linked to a promoter and a nucleic acid sequence encoding a selectable marker operably linked to a promoter; and

(iii) using the selectable marker to isolate cells that produce about 500 ng or greater of said GM-CSF/ $10^6$  cells/24 hours.

23. The method of claim 22, wherein said selectable marker is hygromycin resistance.

24. The method of claim 23, wherein the modified human cell line is cultured in culture medium comprising about 400  $\mu$ g or greater hygromycin/ml culture medium.

25. The method of claim 24, wherein the modified human cell line is subsequently cultured in culture medium comprising about 1,000  $\mu$ g or greater hygromycin/ml culture medium.

26. The method of claim 24, wherein said culture medium is defined.

27. The method of claim 25, wherein said culture medium is defined.

28. The method of claim 22, wherein the promoter to which the nucleic acid sequence encoding GM-CSF is operably linked is a cytomegalovirus promoter.

40. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 17, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated, whereupon administration of said composition, an immune response to said cancer is stimulated.

41. The method of claim 40, wherein said cancer antigen is a cell of said cancer.

42. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 19, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated, whereupon administration of said composition, an immune response to said cancer is stimulated.

43. The method of claim 42, wherein said cancer antigen is a cell of said cancer.

44. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 20, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated, whereupon administration of said composition, an immune response to said cancer is stimulated.

45. The method of claim 44, wherein said cancer antigen is a cell of said cancer.

46. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 21, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated, whereupon administration of said composition, an immune response to said cancer is stimulated.

47. The method of claim 46, wherein said cancer antigen is a cell of said cancer.

50. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 17, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated.

51. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 19, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated.

52. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 20, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated.

53. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 21, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated.